Arch Orthop Trauma Surg (2005) 125: 51–55
DOI 10.1007/s00402-004-0760-8

ORIGINAL ARTICLE

**Anders Björkman · Isabella M. Burtscher
Peter J. Svensson · Andreas Hillarp · Jack Besjakov
Göran Benoni**

Factor V Leiden and the prothrombin 20210A gene mutation and osteonecrosis of the knee

Received: 17 May 2004 / Published online: 18 November 2004

Abstract *Introduction* The pathogenesis behind osteonecrosis of the knee is still unknown. Circulatory impairment of the bone secondary to thrombosis in the microcirculation has been suggested as a mechanism. The purpose of this study was to examine the association between osteonecrosis of the knee and abnormalities in the thrombotic pathway in the form of factor V Leiden and the prothrombin 20210A gene mutation. *Materials and methods* Thirty-eight consecutive patients (13 men and 25 women) with osteonecrosis of the knee without a history of knee trauma or surgery to the knee were enrolled in this study. Assays for the detection of factor V Leiden and the prothrombin 20210A gene mutation were performed, and the results were compared with those from 282 healthy volunteers. *Results* Six patients were diagnosed with secondary osteonecrosis, four corticosteroid-induced and two alcohol-induced. In 32 patients, no aetiological factor was found, and these patients were diagnosed with primary osteonecrosis of the knee. Twelve patients had 14 gene mutations, 11 factor V Leiden and 3 prothrombin 20210A gene mutations. Factor V Leiden and the prothrombin 20210A gene mutation occurred significantly ($p = 0.006$) more frequently in patients with osteonecrosis than in a population of 282 healthy volunteers (odds ratio 3.1, 95%CI 1.4–6.6). *Conclusion* The results of this study suggest that coagulation abnormalities in the form of factor V Leiden and the prothrombin 20210A gene mutation might play a role in osteonecrosis of the knee.

Keywords Osteonecrosis · Knee · Factor V Leiden · Prothrombin gene

A. Björkman · G. Benoni
Department of Orthopaedics, University Hospital Malmö, 20502 Malmö, Sweden

I. M. Burtscher
Department of Radiology, University Hospital Lund, 22185 Lund, Sweden

P. J. Svensson
Department of Coagulation Disorders, University Hospital Malmö, 20502 Malmö, Sweden

A. Hillarp
Department of Clinical Chemistry, University Hospital Malmö, 20502 Malmö, Sweden

J. Besjakov
Department of Radiology, University Hospital Malmö, 20502 Malmö, Sweden

A. Björkman (✉)
Department of Hand Surgery, University Hospital Malmö, 20502 Malmö, Sweden
E-mail: anders.bjorkman@hand.mas.lu.se
Tel.: +46-40-331000
Fax: +46-40-928855

Introduction

Spontaneous osteonecrosis of the femoral condyles has been recognised since 1968, when it was first described by Ahlbäck et al. [2]. The knee is the second most common location for osteonecrosis, although osteonecrosis is 10 times more common in the femoral head [20]. Osteonecrosis of the knee is most commonly found in the medial femoral condyle, but it can also occur in the lateral femoral condyle and in the tibia plateau [6]. Osteonecrosis of the knee is the cause of 1% of knee arthroplasties performed in Sweden [24].

Traditionally, osteonecrosis of the knee has been divided into spontaneous or primary, which occurs in the elderly patient without associated risk factors, and atraumatic or secondary osteonecrosis seen predominantly in the young patient in association with corticosteroid medication or alcohol abuse [19].

In the early 1990s, disturbed blood supply and a hypercoagulable state were suggested as risk factors for osteonecrosis [14]. Although the pathogenesis of osteonecrosis of the knee remains unknown, an impairment of the blood supply to the knee has been suggested as a potential cause, possibly due to injury to the vessel wall as well as venous occlusion [27].

At present, it is thought that patients with idiopathic osteonecrosis of the hip most likely have some type of coagulation abnormality that has not been identified [16]. Thrombophilia, an increased tendency to form thrombi, commonly caused by factor V Leiden and the prothrombin 20210A gene mutation, has been implicated as a potential pathogenic factor of non-traumatic osteonecrosis of the hip [9]. Resistance to activated protein C (APC) is the most common genetic defect observed in patients with thrombosis, particularly in those with familial thrombosis [25]. The genotype of APC resistance has been shown to be based on the mutation of Arg to Gln in factor V at position 506 (factor V Leiden) [5]. The risk of thrombosis due to APC resistance is increased approximately 5- to 10-fold in the heterozygous state, and about 50- to 100-fold in the homozygous state [29]. In the general population, the frequency of factor V Leiden varies between 1% and 10% depending on the geographic location [23]. The population in southern Sweden, where this study was performed, has a frequency of 10%, which is one of the highest frequencies in the world [26].

A mutation in the prothrombin gene, the substitution of G for A at nucleotide position 20210, which results in increased plasma prothrombin levels, has also been reported to be associated with the occurrence of venous thrombosis [22]. The frequency of the prothrombin 20210A gene mutation is 6%–12% in patients with deep venous thrombosis compared with 1%–4% in the general population [7].

As thrombophilia has been suggested as a cause of osteonecrosis of the hip, abnormalities in the thrombotic pathway are a likely cause of osteonecrosis of the knee. However, to our knowledge, no studies have provided evidence of a role of thrombophilia in this condition. The current study investigated the association of factor V Leiden and the prothrombin 20210A gene mutation with osteonecrosis of the knee.

Patients and methods

A series of consecutive patients with osteonecrosis of the knee diagnosed between 1998 and 2002 at our institute and without a history of knee trauma or surgery to the knee prior to their osteonecrosis were prospectively enrolled in this study. Forty-four patients were invited to participate in the study: 38 enrolled, and 6 declined. At diagnosis, all patients underwent a complete clinical and radiographic evaluation and blood sampling. In the clinical evaluation, special attention was paid to corticosteroid medication, alcohol abuse, and history of thromboembolic events. An aetiological factor for the osteonecrosis was identified for each patient. To be classified as having a corticosteroid-induced osteonecrosis, patients had to have been taking continuous corticosteroid medication of more than 20 mg/day of prednisone for a minimum of 2 months [9] prior to symptoms arising in the knee. To be classified as having an alcohol-induced osteonecrosis, patients had to have had an estimated regular alcohol consumption of more than 400 ml per week [12] prior to symptoms arising in the knee. In patients with no obvious underlying aetiology, the disease was classified as primary osteonecrosis.

To verify the diagnosis of osteonecrosis and to discover asymptomatic osteonecrosis in the contralateral knee, radiographs and MRI scans were reviewed by two radiologists for findings in accordance with osteonecrosis of the knee. Both knees were examined by plain X-rays in all patients. MRI scans were available for 21 patients and bilateral MRI for 15 of them. The location of the osteonecrosis was noted as well as other pathological findings.

Factor V Leiden and the prothrombin 20210A gene mutation were identified as previously described [22, 29]. The control group comprised 282 healthy volunteers, 180 men and 102 women, mean age 59 ± 12 years (range 34–86 years), with no history of thrombosis and from the same geographic area as the study subjects [11, 26]. The control group was used for estimation of the frequency of factor V Leiden and the prothrombin 20210A gene mutation in the general population.

Furthermore, analysis of the blood group and a basic coagulation profile including haemoglobin concentration, platelets, prothrombin time and activated partial thromboplastin time were done.

The local Ethics Committee approved the study design, and written informed consent was obtained from all patients.

Statistical analysis

The odds ratios and 95% confidence intervals (CI) for the genetic aberrations were calculated according to Kirkwood [15].

Results

Thirty-eight patients (13 men and 25 women) with osteonecrosis of the knee were included in the study. Mean age at diagnosis was 67 years (range 26–88 years), lower ($p = 0.057$; t-test) in men (61 years, range 26–78 years) than in women (70 years, range 38–88 years). Based on data from interviews and clinical examinations, the 38 patients were assigned to aetiological groups. Corticosteroid-induced osteonecrosis was found in 1 man and 3 women treated for inflammatory bowel disease, asthma, rheumatoid arthritis and polymyalgia rheumatica. The mean age in these four patients was 51 years (range 38–68 years). Two men, aged 58 and 59 years, were diagnosed with alcohol-induced osteonecrosis. In 32 patients, 10 men and 22 women, mean age 70 years (range 26–88 years), no aetiological factor could be found, and they were diagnosed with primary osteonecrosis.

A total of 52 osteonecrotic lesions were found in the 38 patients (Table 1). In primary osteonecrosis the

majority of osteonecrotic lesions were seen in the medial femoral compartment, whereas corticosteroid-induced lesions showed multiple condylar involvement affecting the femoral and tibial condyles as well as the patella. Five patients had bilateral osteonecrosis of the knee, verified by MRI, and data on aetiology, multifocality, symptomatology and coagulation disorders for these patients are given in Table 2. Two patients, one with primary and one with corticosteroid-induced osteonecrosis of the knee, had been diagnosed with additional non-traumatic osteonecrosis of the femoral head.

In the current study 12 of 38 patients (32%) had factor V Leiden or the prothrombin 20210A gene mutation or both (Table 3). All patients were heterozygous for the gene mutations. This frequency is significantly higher than the frequency found among 282 healthy volunteers from the same geographic area, with 32 factor V Leiden and 5 prothrombin 20210A gene mutations in 37 volunteers [11, 26] (odds ratio 3.1, 95%CI 1.4–6.6).

Haemoglobin concentration, platelets, prothrombin time and activated partial thromboplastin time were within normal limits in all patients. The frequency of different blood groups among the 38 patients with osteonecrosis did not differ from the general population.

Five patients in the study had a history of deep venous thrombosis or pulmonary embolism, and one of them had factor V Leiden, while one had both gene mutations. The latter patient suffered a deep venous thrombosis after a knee arthroplasty, whereas thromboembolic events in the other four patients were not related to surgery.

Discussion

We evaluated the association between factor V Leiden and the prothrombin 20210A gene mutation and osteonecrosis of the knee in 38 consecutive patients. Results of the current study showed a significant increase in the prevalence of factor V Leiden and the prothrombin 20210A gene mutation among patients with osteonecrosis of the knee in comparison with healthy volunteers.

Since our material comprised consecutive patients diagnosed with osteonecrosis of the knee, no selection bias is thought to exist, and the material represents a true sample of patients with osteonecrosis of the knee in a southern Swedish population.

The distribution of aetiological factors varies among different studies [1, 19, 20, 21]. Our material showed a high frequency of primary osteonecrosis of the knee compared with very few secondary osteonecroses. These results are comparable to the findings of Aglietti et al. [1] reporting on a series of 91 patients with idiopathic osteonecrosis of the knee, average age 66 years. In this series 3 patients had been on steroid medication, and 1 patient had been diagnosed with alcohol abuse. However, in a study by Mont et al. [19], including only patients less than 55 years of age, 123 of 136 patients (90%) with a mean age of 36 years had a history of corticosteroid medication. In a second study, comprising 30 patients operated on with total knee arthroplasty for osteonecrosis and with a mean age of 54 years, Mont et al. [21] found 22 corticosteroid-induced osteonecroses and 8 primary osteonecroses.

It is possible that we have underestimated the incidence of alcohol-induced osteonecrosis due to the difficulties of identifying alcohol abuse.

Although the pathogenesis of osteonecrosis is unknown, vascular insufficiency with damage to the microcirculation resulting in bone ischaemia and subsequent necrosis has been suggested as a pathogenic factor. This vascular insufficiency might be caused by microfractures [17, 28] of osteoporotic subchondral bone, leading to the passage of fluid through the articular cartilage into the subchondral bone and to

Table 1 Location and aetiology of osteonecrosis in the knee: number of lesions

Location		Total	Aetiology		
			Primary	Corticosteroid-induced	Alcohol-induced
Femur	Medial compartment	33	29	3	1
	Lateral compartment	11	6	4	1
Tibia	Medial compartment	4	1	3	-
	Lateral compartment	2	-	2	-
Patella		2	-	2	-
Total		52	36	14	2

Table 2 Bilateral osteonecrosis of the knee

Case	Aetiology	Multifocal osteonecrosis in the knee	Osteonecrosis in other location than knee	Symptoms	Coagulation disorder
1	Primary	No	No	Unilateral	No
2	Primary	No	No	Unilateral	No
3	Corticosteroid-induced	Unilateral	No	Unilateral	No
4	Corticosteroid-induced	Bilateral	Hip	Bilateral	Protrombin gene mutation
5	Corticosteroid-induced	Bilateral	No	Unilateral	No

Table 3 Aetiologic groups and occurrence of factor V Leiden and prothrombin 20210A gene mutation: number of patients (men; women)

Aetiologic groups	Total	Gene mutation	
		Factor V Leiden	Prothrombin 20210A gene
Primary	32 (10; 22)	11[a](4; 7)	2[a](2; 0)
Corticosteroid-induced	4 (1; 3)	0	1 (1; 0)
Alcohol-induced	2 (2; 0)	0	0
Total	38 (13; 25)	11[a](4; 7)	3[a](3; 0)

[a]Two patients had both factor V Leiden and prothrombin 20210A gene mutation

increased bone marrow pressure and deterioration of the microcirculation, or by a venous occlusion induced by a hypercoagulable state. A hypercoagulable state has been suggested as a factor behind osteonecrosis of the hip in adults [9] and in children in some [4, 8] but not all studies [10, 13]. No previous studies have provided evidence of a hypercoagulable state in osteonecrosis of the knee. However, increased intraosseous pressure and venous stasis, a probable result of disturbed microcirculation, have been found by Uchio et al. [27]. They used venography to show venous stasis in the bone marrow and increased intraosseous pressure within the medullary canal, indicating an injury to a vessel or venous occlusion, but they were not able to find actual thrombi. Venous occlusion has also been suggested as a cause of osteonecrosis in general [18] and of osteonecrosis of the knee by Arnoldi et al. [3], who suggested that obstruction of venous drainage could lead to increased intraosseous pressure and consequent necrosis of the bone. In addition, several studies have shown a hypercoagulable state and a significant increase of venous thrombosis among patients with factor V Leiden and the prothrombin 20210A gene mutation [11, 22, 25, 26]. The hypercoagulable state induced by these gene mutations might increase the risk of thrombosis in the small vessels around the knee or in the bone marrow.

This is, to our knowledge, the first study to show a significant association between osteonecrosis of the knee and the presence of factor V Leiden and the prothrombin 20210A gene mutation or both. In a population with a high prevalence of these mutations, our results suggest that these genetic aberrations might outnumber previously accepted aetiological factors such as corticosteroid medication or alcohol abuse as causative agents for osteonecrosis of the knee.

Acknowledgements This study was supported by grants from Herman Järnhardts Foundation. The above study complies with the current laws of Sweden.

References

1. Aglietti P, Insall JN, Buzzi R, Deschamps G (1983) Idiopathic osteonecrosis of the knee. Aetiology, prognosis and treatment. J Bone Joint Surg Br 65:588–597
2. Ahlbäck S, Bauer GCH, Bohne WH (1968) Spontaneous osteonecrosis of the knee. Arthritis Rheum 11:705–733
3. Arnoldi C, Lemperg R, Linderholm H (1975) Intraosseous hypertension and pain in the knee. J Bone Joint Surg Br 57:360–363
4. Arruda VR, Belangero WD, Ozelo MC, Oliveira GB, Pagnano RG, Volpon JB, Annichino-Bizzacchi JM (1999) Inherited risk factors for thrombophilia among children with Legg-Calvé-Perthes disease. J Pediatr Orthop 19:84–87
5. Bertina RM, Koeleman BPC, Koster T, Rosendaal FR, Dirven RJ, Ronde H de, Velden PA van der, Reitsma PH (1994) Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 369:64–67
6. Ecker ML, Lotke PA (1994) Spontaneous osteonecrosis of the knee. J Am Acad Orthop Surg 2:173–178
7. Emmerich J, Rosendaal FR, Cattaneo M, Margaglione M, De Stefano V, Cumming T, Arruda V, Hillarp A, Reny JL (2001) Combined effect of factor V Leiden and prothrombin 20210A on the risk of venous thromboembolism: pooled analysis of 8 case-control studies including 2310 cases and 3204 controls. Thromb Haemost 86:809–816
8. Glueck CJ, Brandt G, Gruppo R, Crawford A, Roy D, Tracy T, Stroop D, Wang P (1997) Resistance to activated protein C and Legg-Perthes disease. Clin Orthop 338:139–152
9. Glueck CJ, Freiberg RA, Fontaine RN, Tracy T, Wang P (2001) Hypofibrinolysis, thrombophilia, osteonecrosis. Clin Orthop 386:19–33
10. Hayek S, Kenet G, Lubetsky A, Rosenberg N, Gitel S, Wientroub S (1999) Does thrombophilia play an aetiological role in Legg-Calvé-Perthes disease? J Bone Joint Surg Br 81:686–890
11. Hillarp A, Zöller B, Svensson PJ, Dahlbäck B (1997) The 20210A allele of the prothrombin gene is a common risk factor among Swedish outpatients with verified deep venous thrombosis. Thromb Haemost 78:990–992
12. Hirota Y, Hotokebuchi T, Sugioka Y (1997) Idiopathic osteonecrosis of the femoral head: nationwide epidemiologic studies in Japan. In: Urbaniak JR, Jones JP Jr (eds) Osteonecrosis: etiology, diagnosis and treatment. American Academy of Orthopaedic Surgeons, Rosemont, pp 51–58
13. Hresko T, McDougall PA, Gorlin JB, Vamvakas EC, Kasser JR, Neufeld EJ (2002) Prospective reevaluation of the association between thrombotic diathesis and Legg-Perthes disease. J Bone Joint Surg Am 84:1613–1618
14. Jones JP Jr (1992) Intravascular coagulation and osteonecrosis. Clin Orthop 277:41–53
15. Kirkwood BR (1988) Cohort and case-control studies. In: Kirkwood BR (ed) Essentials of medical statistics. Blackwell Scientific Publications, Oxford, pp 177–183
16. Lieberman JR, Berry DJ, Mont MA, Aaron RK, Callaghan JJ, Rayadhyaksha A, Urbaniak JR (2002) Osteonecrosis of the hip: management in the twenty-first century. J Bone Joint Surg Am 84:834–853
17. Lotke PA (1997) Tibial osteonecrosis. In: Urbaniak JR, Jones JP Jr (eds) Osteonecrosis: etiology, diagnosis and treatment. American Academy of Orthopaedic Surgeons, Rosemont, pp 425–428
18. Mankin HJ (1992) Nontraumatic necrosis of bone (osteonecrosis). N Engl J Med 326:1473–1479
19. Mont MA, Baumgarten KM, Rifai A, Bluemke DA, Jones LC, Hungerford DS (2000) Atraumatic osteonecrosis of the knee. J Bone Joint Surg Am 82:1279–1290
20. Mont MA, Myers TH, Krackow KA, Hungerford DS (1997) Total knee arthroplasty for corticosteroid associated avascular necrosis of the knee. Clin Orthop 338:124–130
21. Mont MA, Rifai A, Baumgarten KM, Sheldon M, Hungerford DS (2002) Total knee arthroplasty for osteonecrosis. J Bone Joint Surg Am 84:599–603
22. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM (1996) A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 88:3698–3703

23. Rees DC, Cox M, Clegg JB (1995) World distribution of factor V Leiden. Lancet 346:1133–1134
24. Robertsson O, Dunbar MJ, Knutsson K, Lidgren L (2000) Past incidence and future demand for knee arthroplasty in Sweden. A report from the Swedish knee arthroplasty register regarding the effect of past and future population changes on the number of athroplasties performed. Acta Orthop Scand 71:376–380
25. Svensson PJ, Dahlbäck B (1994) Resistance to activated protein C as a basis for venous thrombosis. N Engl J Med 330:517–522
26. Svensson PJ, Zöller B, Mattiasson I, Dahlbäck B (1997) The factor VR506Q mutation causing APC resistance is highly prevalent amongst unselected outpatients with clinically suspected deep venous thrombosis. J Int Med 241:379–385
27. Uchio Y, Ochi M, Adachi N, Nishikori T, Kawasaki K (2001) Intraosseous hypertension and venous congestion in osteonecrosis of the knee. Clin Orthop 384:217–223
28. Yamamoto T, Bullough PG (2000) Spontaneous osteonecrosis of the knee: the result of subchondral insufficiency fracture. J. Bone Joint Surg Am 82:858–866
29. Zöller B, Svensson PJ, He X, Dahlbäck B (1994) Identification of the same factor V gene mutation in 47 out of 50 thrombosis-prone families with inherited resistance to activated protein C. J Clin Invest 94:2521–2524